510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION

DECISION SUMMARY

A. 510(k) Number:

k120817

B. Purpose for Submission:

New Device

C. Measurand:

IgA antibody to Cardiolipin (CL)
IgA antibody to Beta-2-Glycoprpotein I (β2GPI)

D. Type of Test:

Semi-quantitative chemiluminescent immunoassay (CIA)

E. Applicant:

INOVA Diagnostics, Inc.

F. Proprietary and Established Names:

QUANTA Flash β2GP1 IgA
QUANTA Flash aCL IgA
QUANTA Flash β2GP1 IgA Controls
QUANTA Flash aCL IgA Controls

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5660 – Multiple autoantibodies immunological test system
   21 CFR §862.1150 – Calibrator
   21 CFR § 862.1660 – Quality Control Material (assayed and unassayed)

2. Classification:

   Class II (Assays, Calibrator)
   Class I (Controls)

3. Product code:

   MID, System Test, Anti-Cardiolipin Immunological
   MSV, Antibodies, β2- Glycoprotein I (β2-GPI)
   JJX, Single (specified) Analyte Controls (Assayed and Unassayed)

4. Panel:

   Immunology (82) (Assays)
   Chemistry (75) (Controls and Calibrators)

H. Intended Use:

1. Intended use(s):

   QUANTA Flash β2GP1 IgA: Fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-β2 glycoprotein-1 (β2GP1) IgA antibodies in human citrated plasma and serum on the BIO-FLASH® instrument, as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome, when used in conjunction with other laboratory and clinical findings.

   QUANTA Flash β2GP1 IgA Controls: The QUANTA Flash β2GP1 IgA Controls are intended for the quality control purposes of the QUANTA Flash β2GP1 IgA assay performed on the BIO-FLASH® instrument.

   QUANTA Flash aCL IgA: Fully automated chemiluminescent immunoassay for the semi-quantitative measurement of anti-cardiolipin (aCL) IgA antibodies in human citrated plasma and serum on the BIO-FLASH® instrument, as an aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings.

   QUANTA Flash aCL IgA Controls: The QUANTA Flash aCL IgA Controls are intended for quality control purposes of the QUANTA Flash aCL IgA assay performed on the BIO-FLASH® instrument.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For Prescription Use only

4. Special instrument requirements:

   BIO-FLASH® Instrument System (k083518)

I. Device Description:

QUANTA Flash β2GP1 IgA Kit:

1. QUANTA Flash β2GP1 IgA Kit Reagent Pack (cartridge): One cartridge contains sufficient material for 50 tests. Each reagent pack contains the following sealed reagent tubes:
   a. Microparticle Reagent: 1 vial of β2GP1 (purified human) coated magnetic particles preserved in buffer solution.
   b. Assay Buffer: 1 vial of Tris-buffered saline with protein stabilizers and surfactant. Preservatives: sodium azide and chloramphenicol.
   c. Tracer IgA: 1 vial of isoluminol conjugated monoclonal anti-human IgA antibodies in phosphate buffered saline with protein (bovine) stabilizer. Preservative: sodium azide.
   d. 1 vial of sample diluent used for the regular predilution of the sample and automatic dilution in the rerun.

2. β2GP1 IgA Calibrator 1: 1 x 1 mL barcoded tube of a solution with human anti-β2GP1 IgA in a phosphate buffer, containing bovine serum albumin, stabilizers and preservative. Each vial contains sufficient material for 10 uses.
3. β2GP1 IgA Calibrator 2: 1 x 1 mL barcoded tube of a solution with human anti-β2GP1 IgA in a phosphate buffer, containing bovine serum albumin, stabilizers and preservative. Each vial contains sufficient material for 10 uses.

QUANTA Flash β2GP1 IgA Control Kit: Each control kit contains three vials each of Low Control and High Control. Control material contains human antibodies to β2GP1 in a Tris-buffered saline solution with chloramphenicol and sodium azide. Each vial contains sufficient material for at least 25 uses.

QUANTA Flash aCL IgA Kit:

1. QUANTA Flash aCL IgA Kit contains one reagent pack (cartridge) with sufficient material for 50 tests. Each reagent pack contains the following sealed reagent tubes:
    a. Microparticle Reagent: 1 vial of cardiolipin coated magnetic particles preserved in buffer solution.
    b. Assay Buffer: 1 vial of Tris-buffered saline with protein stabilizers and surfactant. Preservatives: sodium azide and chloramphenicol.
    c. Tracer IgA: 1 vial of isoluminol conjugated monoclonal anti-human IgA antibodies in phosphate buffered saline with protein (bovine) stabilizer. Preservative: sodium azide.
    d. 1 vial of sample diluent used for the regular predilution of the samples and automatic dilution in the rerun.
2. aCL IgA Calibrator 1: 1 x 1 mL barcoded tube of a solution with human anti-aCL IgA in a phosphate buffer, containing bovine serum albumin, stabilizers and preservative. Each vial contains sufficient material for 10 uses.
3. aCL IgA Calibrator 2: 1 x 1 mL barcoded tube of a solution with human anti-aCL IgA in a phosphate buffer, containing bovine serum albumin, stabilizers and preservative. Each vial contains sufficient material for 10 uses.

QUANTA Flash aCL IgA Control Kit: Each control kit contains three vials each of Low Control and High Control. Control material contains human antibodies to cardiolipin in a Tris-buffered saline solution with chloramphenicol and sodium azide. Each vial contains sufficient material for at least 25 uses.

For both QUANTA Flash β2GP1 and aCL IgA Kits, the following additional materials are required (available from INOVA Diagnostics, Inc.) but not provided:

a. BIO-FLASH Instrument and Software System
b. BIO-FLASH System Rinse contains four 5 liter bottles of phosphate buffered saline with Tween-20 and sodium azide.
c. BIO-FLASH Triggers contains one bottle each of Trigger 1 (the catalyst) and 2 (the oxidant).

J. Substantial Equivalence Information:

1. Predicate device name(s) and 510(k) number(s)

   QUANTA Lite β2GP1 IgA ELISA, k973006
   QUANTA Lite ACA III IgA ELISA, k953366

2. Comparison with predicate:

QUANTA Flash™ β2GP1 IgA

Similarities		
Item	Device QUANTA Flash β2GP1 IgA	Predicate QUANTA Lite β2GP1 IgA ELISA
Intended Use	Semi-quantitative measurement of anti-β2 glycoprotein-1 (β2GP1) IgA antibodies	Same
Coating Antigen	Purified human β2 GP1	Same
Cutoff	20 CU	Same (20 SAU)
Antigen Detected	Human β2GP1	Same
Results Interpretation	< 20 EU/mL – negative ≥ 20 EU/mL – positive	Same

Differences		
Item	Device QUANTA Flash β2GP1 IgA	Predicate QUANTA Lite β2GP1 IgA ELISA
Indications for Use	Aid in the diagnosis of thrombotic disorders related to primary and secondary antiphospholipid syndrome, when used in conjunction with other laboratory and clinical findings	Used in conjunction with clinical findings and other laboratory tests to aid in the diagnosis of certain autoimmune disease thrombotic disorders, such as those secondary to systemic lupus erythematosus (SLE) or other lupus-like thrombotic diseases
Assay Technology	Chemiluminescent Immunoassay (CIA)	Enzyme-linked Immunosorbent Assay (ELISA)
Solid phase	Antigen coated magnetic particles	Antigen coated wells
Instrumentation	Automated on BIO-FLASH® instrument	Manual
Measuring range	4 – 512 CU	9.375-150 APL
Sample Matrix	Serum and citrated plasma	Serum
Conjugate	Isoluminol conjugated monoclonal anti-human IgA	Horseradish peroxidase conjugated goat anti-human IgA
Signal Detected	Luminescence (visible light)	Absorbance at 450nm
Calibration and unit calculation	Instrument specific working curve based off a 6 point lot specific master curve used for unit calculations; stored on the instrument for the life of the reagent lot	Five point lot specific curve used for unit calculations, run each time the assay is run.
Calibrators	Set of 2: ~ 10 and 100 CU	Set of 5: 150, 75, 37.5, 18.75, 9.375 SAU

QUANTA Flash aCL IgA

Similarities		
Item	Device QUANTA Flash aCL IgA	Predicate QUANTA Lite ACA III IgA ELISA
Intended Use	Semi-quantitative measurement of anti-cardiolipin (aCL) IgA antibodies	Same

Similarities		
Item	Device QUANTA Flash aCL IgA	Predicate QUANTA Lite ACA III IgA ELISA
Analyte Detected	Human IgA anti-cardiolipin antibodies	Same
Controls	One Cardiolipin IgA Positive Control, one Negative Control	Same
Shelf-life	One year	Same

Differences		
Item	Device QUANTA Flash aCL IgA	Predicate QUANTA Lite ACA III IgA ELISA
Indications for Use	Aid in the diagnosis of thrombotic disorders related to primary and secondary anti-phospholipid syndrome (APS), when used in conjunction with other laboratory and clinical findings	Used in conjunction with clinical findings and other laboratory tests to aid in assessing the risk of thrombosis in individuals with systemic lupus erythematosus (SLE) or lupus-like disorders
Assay Methodology	Chemiluminescent Immunoassay (CIA)	Enzyme-linked Immunosorbent Assay (ELISA)
Solid phase	Antigen coated magnetic particles	Antigen coated wells
Instrumentation	Automated on the BIO-FLASH® instrument	Manual
Measuring range	1.4 to 351.6 CU	9.375-150 APL
Sample matrix	Serum and citrated plasma	Serum
Coating antigens	Bovine cardiolipin and purified human β2 GP1	Purified cardiolipin antigen (1,1',2,2' Tetraoleoyl Cardiolipin (sodium salt)) and β2GP1 from human/bovine serum)
Conjugate	Isoluminol conjugated monoclonal anti-human IgA	Horseradish peroxidase conjugated goat anti-human IgA
Signal detected	Luminescence (visible light)	Absorbance at 450 nm
Calibration and unit calculation	Instrument specific working curve based off a 6 point lot specific master curve used for unit calculations; stored on the instrument for the life of the reagent lot.	Five point lot specific curve used for unit calculations, run each time the assay is run.
Calibrators	2 ~ 10 and 80 CU	Set of 5: 150, 75, 37.5, 18.75, 9.375 APL
Cutoff	20 CU	12 APL
Results Interpretation	< 20 EU/mL – negative ≥ 20 EU/mL – positive	< 12 APL – negative 12 – 20 APL – indeterm > 20 APL – positive

K. Standard/Guidance Document Referenced (if applicable):

CLSI EP05-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline, Second Edition.

CLSI EP06-A, Evaluation of the Linearity of Quantitative Measurement Procedures: A Statistical Approach

CLSI EP07-A2, Interference Testing in Clinical Chemistry, Second Edition.

CLSI EP09-A2, Method Comparison and Bias Estimation Using Patient Samples

EP17-A, Protocols for Determination of Limits of Detection and Limits of Quantification, Approved Guideline.

CLSI EP25-A, Evaluation of Stability of In Vitro Diagnostic Reagents; Approved Guideline

L. Test Principle:

The principles of the QUANTA Flash β2GP1 and aCL IgA assays are similar to other solid phase indirect immunosorbent assays. The assays are a chemiluminescent two-step immunoassay consisting of magnetic particles coated with either human β2GP1 or bovine cardiolipin and human purified ß2GP1, which capture, if present either the β2GP1 or the aCL antiphospholipid antibodies from the sample.

The solid phase is paramagnetic beads and the detecting reagent is a mixture of isoluminol-conjugated monoclonal antibodies to human IgA. A patient's serum is diluted with sample dilution buffer in a disposable cuvette. A small amount of this patient dilution is combined with assay buffer and β2GP1 or aCL beads in a second cuvette, and mixed. This reaction cuvette is incubated for 9½ minutes at 37°C. The cuvette is then exposed to a small magnet that holds the beads in place, the liquid is aspirated, and the beads are resuspended as system rinse is added to the cuvette and the magnet is removed. This wash cycle is repeated one more time. During the third wash, no system rinse is added after the aspiration step, rather the isoluminol conjugate (known as Tracer IgA) is added to the beads in the cuvette, and mixed. Again, the cuvette is incubated for 9½ minutes at 37°C. Three wash steps, as described in the first wash step above, are performed on the beads. In the fourth wash step, no liquid is added to the beads after the aspiration.

The cuvette is then placed in a light-tight luminometer and the beads are exposed to a catalyst and an oxidizing agent. These two reagents, or "Triggers", cause the isoluminol to produce a flash of visible light. The light produced from this reaction is measured as Relative Light Units (RLU) by the BIO-FLASH optical system. The RLUs are proportional to the amount of bound isoluminol conjugate, which in turn is proportional to the amount of IgA anti-β2GP1 or anti-aCL antibodies bound to the β2GP1 or cardiolipin on the beads.

The QUANTA Flash β2GP1 and aCL IgA assay utilizes a 4 Parameter Logistic Curve (4PLC) fit data reduction method to generate a Master Curve. The Master Curve is predefined, lot dependent and it is uploaded to the instrument through the reagent cartridge barcode. With the measurement of calibrators, the predefined Master Curve is transformed to a new, instrument specific Working Curve. The concentration values of the calibrators are included in the calibrator tube barcodes.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. Precision/Reproducibility:

   Precision of the QUANTA Flash™ β2GP1 and aCL IgA assays was assessed in accordance with CLSI EP5-A2 document, by running 11-12 serum samples from different parts of the claimed assay ranges in duplicate with two runs per day for at least 20 days (some samples were run for 21 days) for a minimum of 80

measurements (N) per sample. Within-run (repeatability), between-run, between-day and total precision were calculated by the *Analyse-it for Excel* software. The results are summarized in the tables below:

QUANTA Flash™ β2GP1 IgA:

Sample	N	Mean (CU)	Within-Run (Repeatability)		Between-Run		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	84	412.3	27.4	6.7%	26.4	6.4%	21.2	5.1%	43.6	10.6%
2	80	113.8	4.9	4.3%	8.6	7.6%	6.1	5.3%	11.6	10.2%
3	80	109.6	4.3	3.9%	6.9	6.3%	12.1	11.0%	14.6	13.3%
4	84	27.7	1.2	4.5%	2.3	8.3%	1.5	5.4%	3.0	10.9%
5	80	8.3	0.9	11.4%	0.7	8.7%	0.1	1.1%	1.2	14.4%
6	80	7.3	1.1	15.3%	0.0	0.0%	0.8	11.4%	1.4	19.1%
7	80	18.6	0.6	3.0%	1.2	6.7%	0.4	2.2%	1.4	7.6%
8	80	23.1	0.6	2.7%	1.2	5.1%	0.0	0.9%	1.3	5.8%
9	80	20.7	0.6	2.7%	0.5	2.2%	0.8	4.0%	1.1	5.3%
10	80	21.2	0.4	2.0%	0.8	3.5%	0.8	3.7%	1.2	5.5%
11	80	433.4	23.3	5.4%	18.7	3.4%	28.9	6.7%	41.6	9.6%

QUANTA Flash™ aCL IgA:

Sample	N	Mean (CU)	Within-Run (Repeatability)		Between-Run		Between-Day		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	84	247.42	12.6	4.9%	6.4	2.6%	21.9	8.9%	25.9	10.5%
2	84	98.7	2.34	2.4%	3.54	3.6%	6.8	6.9%	8.1	8.2%
3	80	52.7	1.6	3.0%	2.7	5.1%	3.0	5.8%	4.3	8.2%
4	80	24.0	1.1	4.7%	1.3	5.5%	1.5	6.4%	2.3	9.7%
5	80	3.9	0.3	8.5%	0.0	9.4%	0.3	7.0%	0.6	14.9%
6	80	6.9	0.9	13.4%	0.9	13.4%	0.9	12.4%	1.6	22.7%
7	80	2.0	0.3	13.7%	0.3	14.8%	0.0	5.8%	0.4	21.0%
8	80	18.9	0.6	3.2%	0.5	2.6%	0.7	3.5%	1.0	5.4%
9	80	16.5	0.4	2.3%	0.7	4.2%	0.2	1.2%	0.8	5.0%
10	80	23.4	2.2	9.4%	1.5	6.4%	0.0	0.0%	2.7	11.4%
11	80	23.1	0.7	3.2%	0.9	3.8%	0.3	1.3%	1.2	5.1%
12	80	287.7	16.1	5.6%	0.0	0.0%	24.3	8.4%	29.1	10.1%

Lot-to-lot Reproducibility: Patient samples were tested using three different lots of QUANTA Flash β2GP1 or aCL IgA reagent cartridges. Nine patient samples were each tested once across three different production lots and three instruments for a total of nine measurements. An additional two samples close to the cutoff were tested in a separate experiment in duplicate on three different instruments across two lots. Results are summarized below:

QUANTA Flash™ β2GP1 IgA

Sample	Mean (CU)	Lot 1		Lot 2		Lot 3		Lot-to-Lot Variation	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Pt 1	50.9	5.5	10.6%	3.7	7.4%	2.4	4.8%	0.5	1.1%
Pt 2	92.4	5.0	5.6%	6.4	6.7%	6.0	6.5%	3.3	3.6%
Pt 3	48.5	6.2	12.9%	2.8	5.8%	1.5	3.1%	0.1	0.3%
Pt 4	13.5	2.5	20.4%	0.5	3.9%	0.4	3.0%	0.9	6.6%
Pt 5	4.0	0.0	0.0%	0.0	0.0%	0.0	0.0%	0.0	0.0%
Pt 6	125.1	5.6	4.5%	14.9	11.8%	6.7	5.3%	1.3	1.0%
Pt 7	68.7	5.1	7.6%	7.8	11.2%	1.3	1.9%	1.8	2.6%
Pt 8	82.3	6.5	7.8%	6.2	7.4%	2.0	2.6%	2.0	2.5%
Pt 9	60.3	4.8	7.9%	3.9	6.4%	1.7	2.8%	1.1	1.8%
Pt 10	19.3	0.68	3.5%	1.4	7.1%	ND	ND	0.32	1.6%
Pt 11	20.9	1.54	7.1%	1.5	7.4%	ND	ND	1.02	4.9%

ND not tested

QUANTA Flash™ aCL IgA

Sample	Mean (CU)	Lot 1		Lot 2		Lot 3		Lot-to-Lot Variation	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
Pt 1	118.5	11.9	10.1%	6.7	5.7%	7.8	6.4%	2.3	1.9%
Pt 2	86.2	4.5	5.5%	3.8	4.6%	2.4	2.6%	6.7	7.7%
Pt 3	39.2	2.0	5.3%	0.7	1.7%	0.3	0.6%	1.9	4.8%
Pt 4	12	0.8	6.4%	0.4	3.3%	0.4	3.0%	0.4	3.1%
Pt 5	5	0.4	8.5%	0.2	2.9%	0.2	3.5%	0.3	5.3%
Pt 6	101.4	6.0	6.3%	3.2	3.2%	1.0	0.9%	7.8	7.7%
Pt 7	54.3	2.5	4.9%	2.1	3.9%	0.3	0.5%	3.3	6.0%
Pt 8	58.5	3.2	5.4%	1.7	2.8%	3.8	6.6%	0.6	1.0%
Pt 9	43.6	2.0	4.7%	1.1	2.5%	1.1	2.6%	0.2	0.4%
Pt 10	17.3	0.56	3.2%	1.2	7.2%	ND	ND	0.58	3.4%
Pt 11	21.3	0.83	3.8%	1.8	7.5%	ND	ND	0.66	3.1%

ND not tested

b. Linearity/assay reportable range:

The QUANTA Flash™ β2GP1 and aCL IgA assay linearity studies were evaluated in accordance with CLSI EP6-A Guideline. For each assay, 9 serum samples with various β2GP1 IgA or aCL IgA concentrations were diluted by combining the positive serum sample with the negative serum sample. The observed values were graphed against the calculated values and linear regression was performed. The study results are summarized in the tables below:

QUANTA Flash™ β2GP1 IgA:

Sample	Test Range (CU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2
1	51.2 to 512.0	0.98 (0.93 to 1.03)	-4.44 (-20.31 to 11.43)	1.00
2	21.2 to 212.4	0.95 (0.86 to 1.04)	-3.78 (-16.87 to 9.32)	0.99
3	20.4 to 204.6	0.97 (0.92 to 1.01)	8.65 (3.65 to 13.66)	1.00
4	12.8 to 128.1	0.96 (0.89 to 1.02)	5.68 (0.36 to 11.01)	0.99
5	7.5 to 74.7	1.00 (0.78 to 1.22)	-11.21 (-23.36 to 0.94)	0.93
6	1.9 to 19.3	1.32 (1.09 to 1.56)	-7.91 (-11.31 to -4.51)	0.96
7	2.2 to 21.9	0.95 (0.88 to 1.01)	0.72 (-0.2 to 1.64)	0.98
8	2.1 to 20.7	0.96 (0.88 to 1.04)	0.018 (-0.97 to 1.01)	0.97
9	3.17 to 31.7	0.99 (0.95 to 1.03)	-0.56 (-1.30 to 0.19)	0.99

The reportable range of the assay is defined by the lowest and highest points on the master calibration curve. The claimed reportable range for the QUANTA Flash™ β2GP1 IgA assay is from 4 CU to 512 CU.

QUANTA Flash™ aCL IgA:

Sample	Test Range (CU)	Slope (95% CI)	Y-Intercept (95% CI)	R^2
1	35.2 to 351.6	1.00 (0.95 to 1.05)	-1.10 (-12.70 to 10.50)	1.00
2	17.1 to 171.0	0.93 (0.83 to 1.04)	-4.92 (-17.38 to 7.54)	0.98
3	15.9 to 158.7	0.94 (0.91 to 0.98)	5.32 (1.97 to 8.67)	1.0
4	10.3 to 103.5	0.95 (0.93 to 0.98)	3.00 (1.07 to 4.92)	1.0
5	4.7 to 46.7	0.97 (0.76 to 1.17)	-6.78 (-14.13 to 0.57)	0.94
6	1.6 to 16.4	1.03 (0.93 to 1.13)	-1.41 (-2.56 to -0.267)	0.98
7	2.3 to 22.7	1.01 (0.93 to 1.07)	-0.25 (-1.24 to 0.732)	0.98
8	1.4 to 14.3	1.09 (1.01 to 1.16)	-1.11 (-1.17 to -0.46)	0.98
9	3.3 to 33.5	1.05 (1.03 to 1.07	-1.71 (-2.12 to -1.31)	1.00

The reportable range of the assay is defined by the lowest and highest points on the master calibration curve. The claimed reportable range for the QUANTA Flash™ aCL IgA assay is from 1.4 CU to 351.6 CU.

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Traceability:
There is no international or certified reference material available for anti-β2 GP1 or anti-aCL antibodies. The assay is calibrated in relative arbitrary units (CU or U/mL).

Value Assignment:
Control and calibrator materials are manufactured from human serum containing high-titer IgA anti-β2 GP1 antibodies or IgA anti-aCL antibodies obtained from commercially available sources.

The calibrators included in the QUANTA Flash β2 GP1 and QUANTA Flash aCL IgA assays utilize a predefined lot-specific Master Curve that is stored in the reagent pack barcode. The two calibrator values are assigned using in-house standards and a four-parameter master curve. The assignment values of the two calibrators are used to create a lot-specific four-parameter logistic curve, using two stored parameters from the Master Curve and two lot-specific parameters based on the calibrator values.

The QUANTA Flash β2 GP1 IgA Controls and QUANTA Flash aCL IgA Controls are also manufactured by diluting human serum containing high-titer IgA anti-β2 GP1 antibodies or IgA anti-aCL antibodies into buffer. A target CU value is achieved through trial dilutions on a small scale. Once a dilution is selected, the control is bulked, tested, and adjusted. Controls are tested on at least two instruments, on at least two lots of reagent cartridge, in replicates of 10 to determine final value assignment.

Stability:
Stability studies have been performed to support the following claims:

Reagent Pack Stability:
Shelf-life: The reagent pack can be stored, unopened, at 2–8°C for 1 year based on accelerated stability testing (2 weeks at 37°C). Real time stability is ongoing.

Open/On-board/In-use Stability: Opened reagent packs must be stored on-board the instrument. The working curve has been demonstrated to remain stable for 64 days.

Calibrators Stability:
Shelf-Life: Calibrators showed acceptable accelerated stability for 2 weeks at 37°C, translating to at least 1 year of storage at 2–8°C. Real time stability is ongoing.

On-board Stability: On-board stability testing supported that the calibrators may be stored open for up to 8 hours onboard the instrument.

Control Stability:
Shelf-Life: Controls showed acceptable accelerated stability for 2 weeks at 37°C, translating to at least 1 year of storage at 2–8°C. Real time stability is ongoing.

On-board Stability: Open controls may be used up to 15 times, with a maximum of 10 minutes on-board the instrument per use. The total time the control tubes can be used is 2.5 hours or 10 minutes per use.

d. Detection limit:

Limit of Blank (LoB):
For the QUANTA Flash β2GP1 IgA and QUANTA Flash aCL IgA assays, the LoB was determined according to EP17-A by running immunoglobulin stripped serum diluted at the working dilution of 1:5 run twice in replicates of 30 to obtain 60 measurements.

For the QUANTA Flash β2GP1 IgA assay the LoB was determined to be 412 RLU and for the QUANTA Flash aCL IgA assay the LoB was determined to be 561 RLU. The LoB values for both assays are below the bottom limit of the 4 parameter logistic curve that the instrument uses to calculate chemiluminescent units (CU), and therefore cannot be converted into CU.

Limits of Detection (LoD):
For both devices, the LoD was determined using five serum samples that were serially diluted until the RLU values no longer show a decreasing trend. The dilution factor that gave a RLU value that is noticeably above that background noise was chosen for each serum. A direct dilution using that dilution factor was made for each serum so they were used as the low-level samples that are in between the LoB to approximately 4 x LoB. These 5 samples were run in replicates of 4 for 4 days; 2 days on 1 instrument and 2 more days on another instrument to obtain 16 measurements per sample for a total 80 measurements. It was determined consistent with CLSI EP17-A with proportions of false positives (alpha) less than 5% and false negatives (beta) less than 5%. The LoD for each assay is below the lower limit of the reportable range.

For the QUANTA Flash β2GP1 IgA assay the LoD was determined to be 522 RLU and for the QUANTA Flash aCL IgA assay the LoB was determined to be 812 RLU. The LoD values for both assays are below the bottom limit of the 4 parameter logistic curve (CU)(4850 RLU and 4400 RLU for β2GP1 and aCL respectively)that the instrument uses to calculate chemiluminescent units, and therefore cannot be converted into CU. The LoD for each assay is below the lower limit of the reportable range.

e. Analytical specificity:

Interfering Substances:
Four β2GP1 IgA and aCL IgA serum samples with different analyte concentrations (negative, positive and two around cutoff) were mixed with known quantities of hemoglobin (2, 1, 0.5 mg/mL), bilirubin (1, 0.5, 0.25 mg/mL), cholesterol (224, 112, 56 mg/dL) triglycerides (1000, 500, 250 mg/dL) or rheumatoid factor (about 500, 300, or 100 IU/mL) or buffer. The sera were run in triplicate. The average percent recovery at each dilution is calculated.

No interference was observed up to the concentrations listed in the table below:

Potential Interferent	Final Concentraton Tested
Hemoglobin	200 mg/dL
Bilirubin	100 mg/dL
Cholesterol	224 mg/dL
Triglycerides	1000 mg/dL
Rheumatoid Factor	500 IU/mL

A statement indicating that lipemic and/or grossly hemolyzed sera should not be used is included in the direction insert.

Cross-Reactivity:
A study separate from the clinical sample population was used to determine cross reactivity. Seventy-nine (79) patient samples with various antibodies to autoimmune or infectious diseases were tested in the QUANTA Flash™ β2 GP1 and aCL IgA assays. For infectious disease, samples tested included CMV (5), HCV (5), rubella (5), toxoplasmosis (2), and HSV (5). For the other autoimmune disease states, samples from patients with the following antibodies were tested: 10 rheumatoid factor (RF), 9 anti-cyclic citrullinated peptide (CCP), 8 anti-myeloperoxidase (MPO), and 30 extractable nuclear antibodies (ENA). No cross-reactivity was observed

High dose hook effect:
To assess high dose hook effect, two high positive samples above the reportable ranges were used neat or manually diluted 1:2, 1:4, 1:3 and 1:5 and assayed on both the QUANTA Flash β2GP1 IgA assay and the QUANTA Flash aCL IgA assay

Since these samples ran above the top of the standard curve, standard protocol of the machine is to report results as > 351.6 for aCL IgA and > 512 CU for β2GP1 IgA. A formula was used to calculate higher values. Results for β2GP1 IgA, no hook effect was demonstrated up to 823.4 and 1436.0 CU, respectively. Results for aCL IgA, no hook effect was demonstrated up to 917.7 and 1033.0 CU, respectively.

f. Assay cut-off:

224 patient samples were tested on the QUANTA Flash β2GP1 IgA and QUANTA Flash aCL IgA devices. The sample cohort consisted of 196 apparently normal healthy blood donors and 28 HCV antibody positive samples. To establish the cutoff, CLSI C28-A3C was followed along with recommendations of the International Committee in Sydney that the cutoff between negative and positive for anti-phospholipid antibodies should be >99% of a normal population. As a result, the cut-off for both assays was set to 20.0 U/mL. A result below 20.0 U/mL is considered negative, while 20.0 or greater is considered positive.

2. Comparison studies:

a. Method comparison with predicate device:

Samples for method comparison analysis included those samples from the clinical validation study that were within the reportable range of the assays. For the first set of tables for each analyte, only samples within the measuring ranges defined by the lowest and highest calibrators of both the QUANTA Flash and predicate assays are included in this method comparison. This comparison included 148 total patient samples for the QUANTA Flash β2GP1 IgA and 99 samples for QUANTA Flash aCL IgA. The predicate for the QUANTA Flash aCL IgA has an indeterminate zone so data are also analyzed using the intermediate results as positive or negative. Agreement for the entire validation set (n = 632) including samples above or below the assay measuring ranges for each assay are also presented below.

QUANTA Flash β2GP1 IgA:

Samples within both assay measuring ranges (n=148)		Predicate β2GP1 IgA ELISA (9.4 –150 SAU)		
		Positive > 20 SAU	Negative < 20 SAU	Total
QUANTA Flash β2GP1 IgA (4 – 512 CU)	Positive > 20 CU	63	28*	91
	Negative <20 CU	18**	39	57
	Total	81	67	148

*9APS, 4 PAPS, 11 SAPS, 4 SLE.

**7APS, 1PAPS, 7 SAPS, 1 RA, 2 SLE.

Positive Percent Agreement (63/81): 77.8% (95% CI: 67.2–86.3%)
Negative Percent Agreement (39/67): 58.2% (95% CI: 45.5–70.2%)
Overall Percent Agreement (102/148): 68.9%

All samples in Clinical/Validation set (n=632)		Predicate β2GP1 IgA ELISA (9.4 –150 SAU)		
		Positive > 20 SAU	Negative < 20 SAU	Total
QUANTA Flash β2GP1 IgA (4 – 512 CU)	Positive > 20 CU	71	39*	110
	Negative <20 CU	38**	484	522
	Total	109	523	632

*35 APS, 4 SLE.

**23 APS, 1 snAPS, 1 RA, 9 infectious, 4 SLE.

Positive Percent Agreement (71/109): 65.1% (95% CI: 55.4–74.0%)
Negative Percent Agreement (484/523): 92.5% (95% CI: 89.9–94.6%)
Overall Percent Agreement (555/632): 87.8%

QUANTA Flash aCL IgA:

Samples within both assay measuring ranges (n=99)		Predicate aCL IgA ELISA (9.4 – 150 APL)			
		Positive (>20 APL)	Borderline (12– 20 APL)	Negative (< 12 APL)	Total
QUANTA Flash aCL IgA (1.4 – 351.6 CU)	Positive (>20 CU)	23	*21*	8*	52
	Negative (<20 CU)	8**	*18*	21	47
	Total	31	*39*	29	99

*6 APS and 2 SLE
**6 APS, 1 SLE and 1 SLE-like

Samples within both assay measuring ranges Intermediate samples excluded (n=60)		Predicate aCL IgA ELISA		
		Positive (>20 APL)	Negative (< 12 APL)	Total
QUANTA Flash aCL IgA	Positive (>20 CU)	23	8*	31
	Negative (<20 CU)	8**	21	29
	Total	31	29	60

*6 APS and 2 SLE
** 6 APS, 1 SLE, 1 SLE-like

Positive Percent Agreement (23/31): 74.2% (95% CI: 55.4–88.1%)
Negative Percent Agreement (21/29): 72.4% (95% CI: 52.8–87.3%)
Overall Percent Agreement (44/60): 73.3%

Samples within both assay measuring ranges Borderline Samples for Predicate Considered Positive (>12) (n=99)		Predicate aCL IgA ELISA		
		Positive (>12 APL)	Negative (< 12 APL)	Total
QUANTA Flash aCL IgA	Positive (>20 CU)	44	8*	52
	Negative (<20 CU)	26**	21	47
	Total	70	29	99

*6 APS and 2 SLE
** 17 APS, 7 SLE, 1 SLE-like, 1 RA

Positive Percent Agreement (44/70): 62.9% (95% CI: 50.5%–74.1%)
Negative Percent Agreement (21/29): 72.4% (95% CI: 52.8–87.3%)
Overall Percent Agreement (65/99): 65.6%

Samples within both assay measuring ranges Borderline Samples for Predicate Considered Negative (< 20) (n=99)		Predicate aCL IgA ELISA		
		Positive (>20 APL)	Negative (< 20 APL)	Total
QUANTA Flash aCL IgA	Positive (>20 CU)	23	29*	52
	Negative (<20 CU)	8**	39	47
	Total	31	68	99

* 24 APS, 5 SLE
**6 APS, 1 SLE and 1 SLE-like

Positive Percent Agreement (23/31): 74% (95% CI: 55.4% – 88.1%)
Negative Percent Agreement (39/68): 57.4% (95% CI: 44.8% – 69.3%)
Overall Percent Agreement (62/99): 62.6%

All samples In Clinical/Validation set (n=632)		Predicate aCL IgA ELISA (9.4 – 150 APL)			
		Positive (>20 APL)	Borderline (12– 20 APL)	Negative (< 12 APL)	Total
QUANTA Flash aCL IgA (1.4 – 351.6 CU)	Positive	29	21	52*	102
	Negative	12**	26	492	530
	Total	41	47	544	632

* 46 APS, 4 SLE, 1 RA, 1 other Rheumatic Disease
** 8 APS, 2 RA, 1 SLE, 1 SLE-like

All samples In Clinical/Validation set Indeterminate samples excluded (n=585)		Predicate aCL IgA ELISA (9.4 – 150 APL)		
		Positive (>20 APL)	Negative (< 12 APL)	Total
QUANTA Flash aCL IgA (1.4 – 351.6 CU)	Positive (>20 CU)	29	52*	81
	Negative (<20 CU)	12**	492	504
	Total	41	544	585

*46 APS, 4 SLE, 1 RA, 1 other Rheumatic Disease
** 8 APS, 2 RA, 1 SLE, 1 SLE-like

Positive Percent Agreement (29/41): 71% (95% CI: 54.5–83.9%)
Negative Percent Agreement (492/544): 90% (95% CI: 87.7–92.8%)
Overall Percent Agreement (521/585): 89%

All samples In Clinical/Validation set (n=632) Borderline Samples for Predicate Considered Negative (< 20)		Predicate aCL IgA ELISA (9.4 – 150 APL)		
		Positive (>20 APL)	Negative (< 20 APL)	Total
QUANTA Flash aCL IgA (1.4 – 351.6 CU)	Positive (>20 CU)	29	73*	102
	Negative (<20 CU)	12**	518	530
	Total	41	591	632

*64 APS, 7 SLE, 1 RA, 1 Other Rheumatic Disease
** 8 APS, 2 RA, 1 SLE, 1 SLE-like

Positive Percent Agreement (29/41): 70.7% (95% CI: 54.5–83.9%)
Negative Percent Agreement (518/591): 87.6% (95% CI: 84.7–90.2%)
Overall Percent Agreement (547/632): 86.5%

All samples In Clinical/Validation set (n=632) Borderline Samples for Predicate Considered Positive (>12)		Predicate aCL IgA ELISA (9.4 – 150 APL)		
		Positive (>12 APL)	Negative (< 12 APL)	Total
QUANTA Flash aCL IgA (1.4 – 351.6 CU)	Positive (>20 CU)	50	52*	102
	Negative (<20 CU)	38**	492	530
	Total	88	544	632

*46 APS, 4 SLE, 1 RA, 1 Other Rheumatic Diseases
** 22 APS, 3 RA, 8 SLE, 1 SLE-like, 1 syphilis, 1 seronegative SAPS, 1 HCV, 1 HBV

Positive Percent Agreement (50/88): 56.8% (95% CI: 45.8–67.3%)
Negative Percent Agreement (492/544): 90.4% (95% CI: 87.7–92.8%)
Overall Percent Agreement (542/632): 85.8%

b. Matrix comparison:

A matrix comparison study was performed for the QUANTA Flash β2GP1 IgA and QUANTA Flash aCL IgA assays using 42 paired serum and sodium-citrate plasma blood draws for β2GP1 IgA and 49 serum-plasma pairs for aCL IgA. Both sets of samples were within the analytical measuring range of the assays with samples ranging from 4 to 456 CU for β2GP1 IgA and for 1.7 to 329 CU for aCL IgA.

The resulting data support the package insert claim that serum and Na-citrated plasma primary tube specimens are acceptable sample types for use with the QUANTA Flash IgA assays. Results are summarized in table below:

QUANTA Flash β2GP1 IgA:

		Passing/Bablok
Sodium Citrate vs. Serum	N	49
	Range (CU)	4.1 – 456.1 CU
	Slope	1.01
	Y intercept	0.89
	Correlation coefficient (τ)	0.979

QUANTA Flash aCL IgA:

		Passing/Bablok
Sodium Citrate vs. Serum	N	42
	Range (CU)	1.7 – 329 CU
	Slope	1.10
	Y intercept	-2.74
	Correlation coefficient (τ)	0.993

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The validation set consisted of sent of clinically characterized sera from Primary APS, Secondary APS and non-APS diseased controls for a total of 632 patient samples, none of which were used in the training set. The same validation set was used on both IgA aCL and IgA β2GP1. The results of the QUANTA Flash β2GP1 IgA and QUANTA Flash aCL IgA devices in each disease category are shown below:

APS Patient Sub-Group	N	QUANTA Flash β2GP1 IgA % Positive (N)	QUANTA Flash aCL IgA % Positive (N)
Primary APS (PAPS)	85	22.4% (19)	21.2% (18)
Secondary APS (SAPS)	139	36.7% (51)	36.0% (50)
APS not classified as SAPS or PAPS	65	41.5 % (27)	35.4% (23)
Total APS	289	33.2% (97)	31.5% (91)

Non-APS Patient Group	N	QUANTA Flash β2GP1 IgA % Positive (N)	QUANTA Flash aCL IgA % Positive (N)
SLE	119	9.2% (11)	7.6% (9)
APS-like	79*	1.3% (1)	1.3% (1)
Rheumatoid Arthritis	49	2.0% (1)	2.0% (1)
Sjögren's Syndrome	16	0% (0)	0% (0)
Autoimmune Thyroiditis	3	0% (0)	0% (0)
Infectious Diseases	77**	0% (0)	0% (0)
Total	343	3.8% (13)	3.2% (11)

*68 have APS symptoms and 11 have non-APS thrombotic disorders

**2 HSV,1 Lyme disease,2 Rubella,1 CMV,1 Toxoplasmosis, 9 Syphilis, 10 HIV, 21 HCV, 30 HBV

The clinical sensitivity and specificity of the QUANTA Flash β2GP1 IgA and the QUANTA Flash aCL IgA assays are summarized with and without the SLE group in the following table:

Disease	QUANTA Flash β2GP1 IgA		QUANTA Flash aCL IgA	
	Clinical Sensitivity (95% CI)	Clinical Specificity (95%CI)	Clinical Sensitivity (95% CI)	Clinical Specificity (95%CI)
Primary APS (PAPS)	22.4% (14.0–32.7%)	96.2% (93.6–98.0%) 99.1%* (96.8–99.9%)	21.2% (13.1–31.4%)	96.8% (94.3–98.4%) 99.1%* (96.8–99.9%)
Secondary APS (SAPS)	36.7% (28.7–45.3%)	96.2% (93.6– 98.0%) 99.1%* (96.8–99.9%)	36.0% (28.0–44.5%)	96.8% (94.3–98.4%) 99.1%* (96.8–99.9%)
APS not classified as SAPS or PAPS	41.5% (29.4–54.4%)	96.2% (93.6– 98.0%) 99.1%* (96.8–99.9%)	35.4% (23.9–48.2%)	96.8% (94.3–98.4%) 99.1%* (96.8–99.9%)

Disease	QUANTA Flash β2GP1 IgA		QUANTA Flash aCL IgA	
	Clinical Sensitivity (95% CI)	Clinical Specificity (95%CI)	Clinical Sensitivity (95% CI)	Clinical Specificity (95%CI)
Total APS	33.6% (28.1–39.3%)	96.2% (93.6–98.0%) 99.1%* (96.8–99.9%)	31.5% (26.2–37.2%)	96.8% (94.3–98.4%) 99.1%* (96.8–99.9%)

*Specificity without SLE population

b. Other clinical supportive data (when a. is not applicable):
Not applicable.

4. Clinical cut-off**:**

   See Assay Cutoff

5. Expected values/Reference range:

   The expected value in the general population is negative. The normal range was established by testing a population of normal blood donor serum specimens and tested on the QUANTA Flash β2GP1 IgA and QUANTA Flash aCL IgA assays. Results demonstrating incidence in the apparently-disease free samples for this study are provided below:

		QUANTA Flash β2GP1 IgA		QUANTA Flash aCL IgA	
Normal Blood Donors	n	% pos	n	% pos	n
	359	0.56%	2	0.56%	2

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.